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Abbreviations

aa	Amino acid
ABCA1	ATP-binding cassette transporter 1
AChEI	Acetylcholinesterase inhibitor
AD	Alzheimer's disease
ADIT	Alzheimer's disease innovative drug target
AICD	APP intracellular domain
AMPARs	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
AMPS	Ammonium persulfate
ANOVA	Analysis of variance
AP	Alkaline phosphatase
APH-1	Anterior pharyx-defective 1
APLP1	APP-like proteins 1
APLP2	APP-like proteins 2
ApoE	Apolipoprotein
APP	Amyloid protein precursor
ATP	Adenosine-5'-triphosphate
AVE	Anterior visceral endoderm
Aβ	beta-amyloid peptide
BACE-1	beta-site amyloid precursor protein cleaving enzyme 1
BBB	Blood-brain-barrier
Bp	Base pairs
BrdU	Thymidine analog bromodeoxyuridine
BSA	Bovine serum albumin
C°	Degrees Celsius
C1P	Ceramide-1-phosphate
CA1-2-3	Cornu Ammonis 1-2-3
CAA	Cerebral amyloid angiopathy
CamKII	Calmodulin-dependent protein kinase II
CamKII-α	Calmodulin-dependent protein kinase II
cDNA	Complementary DNA
CLU	Clusterin
CNS	Central nervous sytem
COX	Cyclooxygenase
DAPI	4',6-diamidino-2-phenylindole
DDT	Dithiothreitol
DEPC	Diethyl pyrocarbonate
DG	Dentate gyrus
DKK1	Dickkopf- 1
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide
DS	Down Syndrome
DTT	Dithiothreitol
E	Embryonic day
EDTA	Diaminoethanetetra-acetic acid disodium salt
EGFP	Enhanced green fluorescent protein
EOAD	Early-onset Alzheimer's disease
EU	Eoropean Union
EU	European Union
FBS	Foetal bovine serum

FP6	Framework program 6
FTDP-17	Frontotemporal Dementia with Parkinsonism-17
G418	Geneticin selective antibiotic
$GABA_A$	γ-aminobutyric acid
GFAP	Glial fibrillary acidic protein
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GNDF	Glial Cell derived Neurotrophic Factor
G-PCR	G-protein coupled receptor
GSAP	γ-secretase activating proteins
GSK3-α	Glycogen synthase kinase 3 alpha
GSK3-β	Glycogen synthase kinase 3 beta
GWAS	Genome-wide associated studies
hCMV	Human cytomegalovirus
HD	Huntington's disease
HTS	Hit-to Lead Phase
IHC	Immunohistochemistry
IL-1	Interleukin 1
IL-6	Interleukin 6
IL-8	Interleukin 8
Kb	Kilo base
LB	Luria-Bertani
LIF	Leukaemia inhibitor factor
LOAD	Late-onset Alzheimer's disease
LTP	Long term potentation
LTP	Long term potentiation
µg	Microgram
µL	Microlitre
µM	Micrometre
mES	Mouse Embryonic stem cells
MHCII	Major histocompatibility complex class II
mM	Millimolar
mM	Millimolar
mRNA	Messenger ribonucleic acid
MWM	Morris water maze
$NF\text{-}_K\beta$	Nuclear factor kappa beta
NFTs	Neurofibrillary tangles
ng	Nanogram
NGS	Normal goat serum
NMDARs	N-methyl D-aspartate
NSAID	Nonsteroidal anti-inflammatory drugs
p	Probability value
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PBST	PBS with Tween-20
PCR	Polymerase chain reaction
PD	Parkinson's disease
PFA	Paraformaldehyde
PGCs	Primordial germ cells
PHF	Paired helical filament
PICALM	Phosphatidylinositol binding clathrin assembly protein
PKA	Protein kinase A
PP-2A	Protein phosphatase 2
PS1	Presenilin 1

PS2	Presenilin 2
PVDF	Polyvinylidene fluoride
ROS	Reactive oxygen species
RT	Room temperature
RT-PCR	Reverse transcription-PCR
rt-TA	Tet on Transactivator
S1P	Sphingosine-1-phosphate
S1P3	Sphingosine 1-phosphate receptors 3
SB	Subiculum
SDS	Sodium dodecyl sulphate
SM	Sphingomyelin
SR	Serum replacement
TBST	Tris buffered saline with Tween-20
tg	Transgenic
Tg	Transgenic
TGFβ	Transforming growth factor beta
TM	Trans menmbrane
Tm	Melting temperature
TNFα	Tumor necrosis factor-alpha
TRE	Tet systems response
tTA	Tet off Transactivator
U	Unit enzyme
UV	Ultraviolet
Volts	Volts
X^2	Chi-squared
τ	Tau

Acknowledgements

I express my gratitude to my supervisor Dr Andrew Ward for giving me the opportunity to join his laboratory where in the last four years I have learned all I know about science. I also owe him my deepest thanks for reviewing this manuscript and for his gentle way of suggesting without telling. I am also indebted to Dr Georg Terstappen for inviting me to join the ADIT project.

I extent my acknowledgement to Dr Kim Moorwood for challenging my critical thinking and for making me realize that passion, planning, open-mindedness, cooperation and objectivity is essential for succeeding in science. I thank Dr Joanne-Stewart-Cox for helping me at my very beginning with the settling in the lab and for her aid together with the one provided by Dr Kim Moorwood in the production of the transgenic mice.

I wish to express my thanks to Prof Fiorella Casamenti and members of her lab, especially Dr Maria Cristina Rosi, Dr Chiara Fiorentini and Dr Cristina Grossi for inviting me to joining their lab where I was introduced to behavioural studies.

A number of other persons helped along the way and I hope I am not leaving anyone out: thanks to Dr Cheney Drew for sharing her knowledge about perfusion; Dr Heather Bone for her suggestions with mES cell culture; Louise Anderson for providing her expertise any time I needed it and to Iryna Withington for helping with tissue processing.

In the “zebramouse” lab I also had the pleasure to work with very bright and passionate colleagues with whom I had discussions about science and everything else. In particular, thanks to: Dr Andrea Weiner and Dr Asha Recino with whom I shared the bright and dark side of the PhD days and who encouraged me to keep on going.

Thanks to Professor Mayford for providing the CamKIIα-tTA mice and to Dr Andrea Caricasole and Dr Giuseppe Pollio (Siena Biotech, Italy) for providing the hDKK1 and hS1P3 plasmids used in this study. Thanks also to Dr Joanne Stewart-Cox for providing the BASP 1 mES cell line and to the project students Helen Mikhasenko and Vanessa DeMello.

If I am going to be Dr. Manfredi it is also thanks to my lovely housemates Sabine Graf and Peter Goodison which contributed to make the writing up stage enjoyable as I would never have imagined.

Abstract

AD is thought to be caused by an abnormal production and aggregation of amyloid-beta (Aβ) peptide. Consequently, precluding the generation of Aβ has been considered as a strategy for AD treatment. Pharmacological compounds reducing Aβ formation have been developed and tested in preclinical and clinical trials and the often promising results obtained in preclinical trials have not been successful reproduced in clinical trials.

With this in mind in 2005 the European Framework Program 6 funded the ADIT project (Design of Small Molecule Therapeutics for the Treatment of Alzheimer Disease Based on the Discovery of Innovative Drug Targets) and this study has been part of this large collaborative study which involved eight institutions across Europe.

The main aim of the ADIT project was to identify new druggable targets for AD drug discovery. The project involved a screen for novel candidate AD target genes, performed by Siena Biotech and involved a number of other collaborating laboratories with roles in validating these targets. Validated targets were those genes: induced in response to Aβ treatment of cultured neurons and with demonstrable neurotoxic activity; induced in the brains of AD patients and in brains from an existing mouse model of AD; deemed most tractable as targets of small molecule inhibitors. Two targets, DKK-1 and S1P3, fulfilled these requirements.

Drug discovery relies on animal models and the aim of this thesis was to develop transgenic mice for the selected targets and to investigate their role in AD pathology. Animal models were generating by pronuclear injection.

Preliminary findings suggest that S1P3 may contribute to the inflammatory process seen in AD. Chronic neuroinflammation is a common characteristic of AD and it may be responsible of the neuronal loss seen in AD. GFAP immunohistochemistry on brains of the S1P3 mice revealed a strong astrocytotic process particularly evident in the hippocampus (mainly in the dentate gyrus) Upregulation of GFAP is commonly accompanied by astrocyte proliferation and activation which leads to the production of pro-inflammarory and cytotoxic cytokines, as well as toxic molecules. A large body of evidence suggests that by transforming from a basal to a reactive state, astrocytes neglect their neurosupportive functions, thus rendering neurons vulnerable to neurotoxins, including proinflammatory cytokines and reactive oxygen species. The S1P3 mouse model represents a model for acquiring more insights into mechanisms of Aβ-mediated toxicity in AD and a target for preventing astrocyte activation.

The characterization of the DKK-1 mouse model is still in its infancy, nevertheless preliminary analysis have already demonstrated that is up-regulation cause Glycogen synthase kinase-3β

(GSK3-β) activation which in turn hyperphosphorylate tau. It is known that hyperphosphorylation of tau is responsible for NFTs formation and DKK-1 inhibition might prevent this process.